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ANNUAL PROGRESS REPORT

Grant: # N00014-93-1-1039

T&amp;T Code: 3412-175

PRINCIPAL INVESTIGATOR: Dr. Nancy J. O'ConnorINSTITUTION: South Carolina Wildlife & Marine Resources DepartmentGRANT TITLE: Completion of Biofouling Research on The Effects of Marine Bacteria on the Attachment of Larval BarnaclesREPORTING PERIOD: 1 July 1993 - 31 May 1994 (11 months)AWARD PERIOD: 1 July 1993 - 30 June 1994

OBJECTIVE: 1) To complete laboratory bioassays examining the effects of the bacteria Pseudomonas fluorescens, Deleya marina, and Alteromonas macleodii on attachment of larvae of the barnacles Balanus amphitrite and B. improvisus; 2) To perform a study of the distribution and abundance of bacteria and their extracellular materials on polystyrene and glass substrata; and 3) to train an additional minority student in all aspects of the research.

APPROACH: Bacterial cells or supernatant from log and stationary phase bacterial cultures are allowed to attach to assay containers (polystyrene petri dishes or borosilicate glass vials), and the attachment of lab-reared barnacle cyprid larvae to treated or control (unfilmed) assay containers is monitored daily. In cell staining assays, bacterial cells from log and stationary phase cultures are allowed to attach to polystyrene petri dishes or glass microscope slides. Attached cells are fixed and stained with crystal violet. Materials from culture supernatants are allowed to attach to polystyrene petri dishes, then carbohydrate components are stained with alcian blue, periodic acid - Schiff's reagent, or peroxidase - labelled lectins (concanavalin A or wheat germ).

ACCOMPLISHMENTS (last 12 months): All larval attachment bioassays were completed and statistically analyzed. Results of assays testing the effects of bacteria on B. amphitrite were inconsistent from one assay to the next; both enhancement and reduction of cyprid attachment to polystyrene and glass substrata occurred. However, results of assays with B. improvisus cyprids were more consistent; cells and exopolymers of all three bacterial species generally reduced cyprid attachment to polystyrene and increased attachment to glass. Bacterial cell staining did not show qualitative patterns in distribution and abundance that could be related to variability in cyprid attachment among assays. Carbohydrates in culture supernatants did not

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stain appreciably with any of the treatments used. In addition, contact angle measurements were made of water droplets on filmed and unfilmed surfaces. Both bacterial cells and culture supernatants altered surface wettability significantly compared to controls. Preliminary field bioassays indicated that wild B. improvisus cyprids respond differently to D. marina biofilms submerged in natural seawater, than do lab-reared cyprids in lab bioassays. Two African-American student volunteers assisted in laboratory studies. One minority employed by the project became proficient in laboratory culture and analytical techniques. In addition, a major minority summer training program, funded by the state, was developed as a result of this and previous ONR-funded research.

DISAPPOINTMENTS: The ideal lectins for binding with carbohydrates in bacterial culture supernatants were not found within the allotted time.

SIGNIFICANCE: Larval attachment assays employing two barnacle species and three bacterial species provide a more comprehensive understanding of the variability in attachment of larval barnacles to substrata with bacterial biofilms. The results should aid biofouling researchers in refining critical assay procedures. Minority training in marine biological and ecological research will be continued by SCWMRD through a summertime, mentor-based research program.

WORK PLAN (NEXT 12 MONTHS): N/A

PUBLICATIONS AND ABSTRACTS (last 11 months):

1. O'Connor, N.J. and D.L. Richardson. Comparative attachment of barnacle cyprids (Balanus amphitrite Darwin, 1854; B. improvisus Darwin, 1854; & B. eburneus Gould, 1841) to polystyrene and glass substrata. Submitted to J. Exp. Mar. Biol. Ecol.
2. O'Connor, N.J. and D.L. Richardson. Effects of bacteria on attachment of barnacle larvae; lab and field studies. Abstract presented at the 1994 Marine Benthic Ecology Meetings, Mystic, CT.
3. O'Connor, N.J. and D.L. Richardson. The influence of surface-associated bacteria on attachment of barnacle cyprids (Balanus amphitrite). In preparation.

## PROJECT HIGHLIGHT

### Objectives

Complete bioassays of larval barnacle attachment to surfaces with marine bacteria

Examine bacteria and their extracellular materials on surfaces

Train a minority student in laboratory procedures

### Accomplishments

Completed and analyzed bioassays examining the effects of 3 bacterial species on attachment of larvae of 2 barnacle species

Stained bacterial cells on polystyrene and glass surfaces

Two African-American student volunteers assisted in laboratory studies

### Significance

Better understanding of variability of attachment of larval barnacles to surfaces with bacterial biofilms

Refinement of biofouling assay procedures

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**ANNUAL REPORT QUESTIONNAIRE**  
(for ONR use only)

**Principal Investigator:** Nancy J. O'Connor  
**Institution:** South Carolina Wildlife & Marine Resources Department  
**Project Title:** Completion of Biofouling Research on the Effects of Marine Bacteria on the Attachment of Larval Barnacles

**Number of ONR supported**

Papers published in refereed journals: 1 submitted  
Papers or reports in non-refereed publications: 0  
Books or book chapters published: 0  
Papers in preparation: 2

**Number of ONR supported patents/inventions**

Filed: 0  
Granted: 0  
Patent name(s) and number(s): \_\_\_\_\_

**HAVE YOU LICENSED TECHNOLOGIES (E.G., SOFTWARE) THAT WERE DEVELOPED WITH ONR SUPPORT? IF SO, PLEASE DESCRIBE ON A SEPARATE SHEET.**

N/A

**HAVE YOU DEVELOPED INDUSTRIAL/CORPORATE CONNECTIONS BASED ON YOUR ONR SUPPORTED RESEARCH? IF SO, PLEASE DESCRIBE ON A SEPARATE SHEET.**

N/A

**Number of presentations:**    Total                      ONR Project

Invited:            0

Contributed:    1                                      1

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**Trainee Data (only for those receiving full or partial ONR support):**

TOTAL      FEMALE      MINORITY      NON-US CITIZEN

**No. Grad. Students:**

**No. Postdoctorals:**

|                     |   |   |   |
|---------------------|---|---|---|
| No. Technicians     | 1 | 1 | 1 |
| No. Undergraduates: | 2 | 2 | 2 |
| No. Volunteers      |   |   |   |

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**AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP (please describe):** The PI accepted a teaching/research position at Biology Department, University of Massachusetts Dartmouth

**Equipment purchased on grant (number and description of items costing >\$1,500):**

N/A